

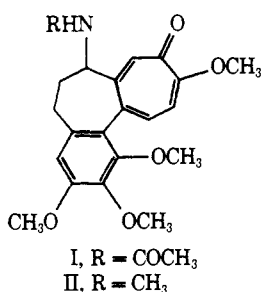
Structure of the Mitotic Spindle Inhibitor Colcemid. *N*-Desacetyl-*N*-methylcolchicine

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Received July 12, 1973

Abstract: Crystals of the mitotic spindle inhibitor *N*-desacetyl-*N*-methylcolchicine (also known as colcemid and demecolcine), $C_{21}H_{25}NO_5$, are orthorhombic, space group $P2_212_1$, $a = 8.686$, $b = 20.24$, $c = 11.168$ Å, $Z = 4$ molecules/cell. The crystal structure has been determined by direct methods and refined by least-squares calculation to an R of 0.072 for 1200 X-ray reflections. The tropolonoid ring is planar with alternating bond lengths. This ring makes a dihedral angle of 54° with the planar benzene ring. The shape of the molecule is found to be similar to that of podophyllotoxin.

The use of *Colchicum* for relief of gout dates back to ancient times.¹ Colchicine (I), the principal active



substance, is known to produce mitotic arrest and has been extensively studied by chemists and biologists.² Colcemid (II), *N*-desacetyl-*N*-methylcolchicine,³ occurs naturally in most of the plants containing colchicine. It has similar antimitotic properties and has been studied as a treatment for chronic myelocytic leukemia⁴ but its high toxicity prevents wide usage. Recent work⁵ indicates that these substances interfere with the growth of microtubules which are widely distributed organelles found in, for example, nerve cells, ciliated cells, leucocytes, and sperm tails. Arrest of mitosis is due to inhibition of the formation of the mitotic spindle, composed of microtubules. While the chemical structures of these compounds are well established, their three-dimensional structures are not, the only crystallographic work being two-dimensional projections of the colchicine-methylene bromide complex.⁶ Since these substances probably interact with microtubule protein which makes up the mitotic spindle, the three-dimensional conformations will be needed to understand their activity. Colcemid was chosen for this X-ray diffraction study because of the ease of obtaining suitable single crystals.

Experimental Section

Crystals of II obtained commercially from CIBA were used without further recrystallization. Intensities were gathered using a

(1) O. J. Eigsti and P. Dustin, Jr., "Colchicine in Agriculture, Biology and Chemistry," The Iowa State College Press, Ames, Iowa, 1955.

(2) G. Deysson, *Int. Rev. Cytol.*, **24**, 99 (1968).

(3) Also called demecolcine and Santavy's Substance F.

(4) A. Burger, Ed., "Medicinal Chemistry," Wiley, New York, N. Y., 1970.

(5) L. Margulis, *Int. Rev. Cytol.*, **34**, 333 (1973).

(6) M. V. King, J. L. de Vries, and R. Pepinsky, *Acta Crystallogr.*, **5**, 437 (1952).

crystal of dimensions $0.3 \times 0.3 \times 0.4$ mm mounted on a Picker full-circle, manual diffractometer with Cu K α (λ 1.54178 Å) X-rays, by the stationary-crystal stationary-counter method. No correction for absorption was made ($\mu r \cong 0.19$). Cell dimensions were calculated from high-order 2θ measurements on a smaller crystal ($0.2 \times 0.2 \times 0.3$ mm). All computing including calculation of diffractometer settings, data reduction, phase determination by direct methods, Fourier series, least-squares refinement, interatomic distances and angles, least-squares planes, and listing of F_o and F_c was done in the author's laboratory on a teletype machine operated in a time-shared mode with a CDC 3800 computer.⁷

Structure Determination. Colcemid, $C_{21}H_{25}NO_5$, forms orthorhombic crystals, space group $P2_212_1$, with $a = 8.686 \pm 0.003$, $b = 20.24 \pm 0.01$, $c = 11.168 \pm 0.003$ Å. With 4 molecules/unit cell all atoms must be in the general position x, y, z ; $x, -y, -z$; $-x, 1/2 - y, 1/2 + z$; $-x, 1/2 + y, 1/2 - z$. The structure was solved by direct phase determination using the program of Syd Hall. The successful starting set of origin and enantiomorph determining phases are:

h	k		ϕ	E
6	11	0	$\pi/2$	5.6
7	14	0	0	3.7
0	19	5	0	2.5
0	12	7	0	2.0

This set gave a scale factor of 0.95 and a value of $\Sigma(|E_o| - |E_c|)/\Sigma E_o = 0.19$. An E map clearly showed all non-hydrogen atoms. The structure was refined in blocks with the least-squares program of Gantzel, Sparks, and Trueblood to a final value of $R = \Sigma|F_o| - |F_c|/\Sigma|F_o| = 0.072$ for 1200 nonzero reflections. The final cycles used anisotropic thermal parameters for oxygen, nitrogen, and carbon atoms. The function minimized was $\Sigma\omega(|F_o| - |F_c|)^2$ with the weight ω equal to $1/\sigma^2$ where σ is the standard deviation of the structure factor estimated from counting statistics and machine instability. Atomic scattering factors were taken from the "International Tables for X-ray Crystallography."⁸ A ΔF map showed most of the hydrogen atoms clearly but they were not included in the calculations. Final atomic parameters are listed in Tables I and II. Distances and angles calculated from these are listed in Tables III and IV. A listing of observed and calculated structure amplitudes will appear in the microfilm edition of this journal.⁹

Results

The molecules (Figure 1) are linked in chains by hydrogen bonds from N to the carbonyl oxygen O(1) with an N-H...O distance of 3.00 Å. The seven-membered tropolonoid ring is planar within 0.02 Å and the benzenoid ring is planar to within 0.03 Å. These two planes form a dihedral angle of 54° . The other

(7) T. N. Margulis, Abstracts, American Crystallographic Association, Summer Meeting, Ottawa, 1970, No. A5.

(8) "International Tables for X-ray Crystallography," Vol. III, Kynoch Press, Birmingham, England, 1962, p 202.

(9) See paragraph at end of paper regarding supplementary material.

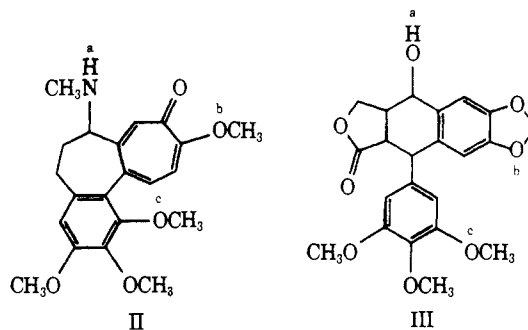
Table I. Aromatic Coordinates^a

Atom	x	y	z
O(1)	0.9545 (6)	0.2447 (3)	0.4921 (5)
O(2)	0.8503 (6)	0.2963 (2)	0.3031 (4)
O(3)	0.2915 (6)	0.4116 (2)	0.5994 (4)
O(4)	0.1296 (5)	0.5048 (3)	0.7247 (5)
O(5)	0.2714 (7)	0.5841 (3)	0.8844 (5)
N	0.7384 (5)	0.2863 (3)	0.8831 (5)
C(1)	0.8549 (8)	0.2882 (3)	0.5081 (7)
C(2)	0.7875 (9)	0.3209 (3)	0.4056 (6)
C(3)	0.6809 (9)	0.3710 (3)	0.4043 (6)
C(4)	0.6055 (8)	0.4042 (3)	0.4997 (7)
C(5)	0.6197 (8)	0.3957 (3)	0.6222 (6)
C(6)	0.7118 (8)	0.3479 (3)	0.6824 (6)
C(7)	0.8116 (9)	0.3026 (3)	0.6326 (6)
C(8)	0.5273 (7)	0.4432 (3)	0.6966 (6)
C(9)	0.5977 (8)	0.4811 (3)	0.7845 (6)
C(10)	0.5166 (9)	0.5297 (4)	0.8481 (7)
C(11)	0.3626 (9)	0.5377 (3)	0.8262 (7)
C(12)	0.2868 (8)	0.4992 (4)	0.7407 (6)
C(13)	0.3700 (8)	0.4509 (3)	0.6804 (6)
C(14)	0.7723 (8)	0.4698 (3)	0.8105 (7)
C(15)	0.7984 (9)	0.4027 (4)	0.8742 (7)
C(16)	0.6969 (8)	0.3467 (3)	0.8203 (6)
C(17)	0.6334 (12)	0.2294 (4)	0.8550 (8)
C(18)	0.2118 (13)	0.3582 (5)	0.6508 (10)
C(19)	0.0910 (10)	0.5608 (5)	0.6476 (10)
C(20)	0.3368 (14)	0.6204 (4)	0.9835 (8)
C(21)	0.8061 (13)	0.3228 (4)	0.1899 (7)

^a Standard deviations estimated from the least-squares calculations, assuming random errors in the intensity data, are given in parentheses as deviations in the last significant figure.

dihedral angle between the normal to the $-C-O-CH_3$ plane and the normal to the benzene or tropolone plane. These angles fall into two groups with the methoxys containing C(18) and C(19) being nearly perpendicular to the benzene ring (82 and 80°, respectively) and the methoxys containing C(20) and C(21) being nearly coplanar with the benzene or tropolone rings (8 and 1°, respectively).

Colchicine and colcemid are believed to act by interfering with the assembly of microtubule protein (tubulin) into microtubules.⁵ In order to see what molecular features are involved it is useful to look at other compounds which behave in the same way. While many substances arrest mitosis (including substances as diverse as ethanol, lead nitrate, and griseofulvin²), certain ones mimic the effects of colchicine closely.

Table II. Anisotropic Thermal Parameters^a $\times 10^4$ in the Form $\text{Exp}[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl)]$

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O(1)	194 (10)	40 (2)	92 (5)	81 (8)	15 (15)	7 (6)
O(2)	178 (9)	30 (1)	74 (5)	43 (7)	19 (12)	-9 (5)
O(3)	131 (8)	31 (2)	86 (5)	-5 (3)	-43 (11)	-11 (6)
O(4)	104 (7)	32 (1)	120 (6)	22 (7)	21 (12)	19 (5)
O(5)	240 (12)	32 (2)	122 (7)	70 (8)	34 (14)	-24 (6)
N	192 (12)	32 (2)	74 (5)	21 (9)	-16 (15)	32 (6)
C(1)	116 (11)	22 (2)	101 (8)	-2 (8)	-15 (18)	-1 (7)
C(2)	142 (11)	18 (2)	59 (6)	1 (8)	-2 (16)	-9 (6)
C(3)	146 (13)	23 (2)	76 (7)	5 (9)	-14 (17)	-1 (6)
C(4)	131 (11)	24 (2)	74 (7)	5 (9)	-6 (17)	-9 (6)
C(5)	101 (10)	18 (2)	78 (7)	-4 (7)	38 (15)	-4 (6)
C(6)	102 (10)	21 (2)	63 (6)	6 (8)	-7 (15)	10 (6)
C(7)	131 (12)	25 (2)	72 (7)	-11 (9)	17 (17)	-2 (6)
C(8)	82 (10)	21 (2)	68 (6)	9 (7)	3 (14)	0 (6)
C(9)	120 (11)	21 (2)	86 (7)	1 (8)	4 (17)	12 (7)
C(10)	140 (13)	24 (2)	90 (8)	16 (9)	39 (17)	2 (7)
C(11)	169 (14)	20 (2)	87 (7)	18 (9)	67 (18)	5 (7)
C(12)	130 (10)	23 (2)	77 (7)	23 (9)	6 (15)	0 (6)
C(13)	140 (12)	22 (2)	74 (7)	14 (8)	7 (16)	32 (6)
C(14)	118 (11)	28 (2)	107 (8)	9 (9)	-13 (18)	-16 (8)
C(15)	138 (12)	32 (2)	84 (8)	9 (10)	-31 (18)	6 (7)
C(16)	115 (11)	21 (2)	74 (7)	10 (8)	-6 (16)	2 (6)
C(17)	268 (18)	27 (2)	147 (11)	-38 (12)	-70 (26)	61 (9)
C(18)	295 (22)	45 (3)	153 (12)	-138 (15)	58 (29)	0 (11)
C(19)	156 (15)	64 (4)	197 (14)	67 (14)	45 (26)	115 (14)
C(20)	389 (25)	38 (3)	102 (9)	69 (16)	-12 (30)	-43 (10)
C(21)	350 (22)	48 (3)	56 (7)	108 (16)	-1 (25)	-4 (8)

seven-membered ring is folded into a boat-like conformation (Figure 2). Overall the structure is consistent with the X-ray work of King, *et al.*⁶ The bond angles and alternating bond lengths in the tropolone ring indicate only partial electron delocalization consistent with recent results on 4-isopropyltropolone.¹⁰

The orientation of the methoxy groups is given by the

(10) J. E. Derry and T. A. Hamor, *J. Chem. Soc., Perkin Trans. 2*, 694 (1972).

In particular, the antineoplastic agent podophyllotoxin (III) behaves remarkably like colchicine in that it (1) causes "C-mitosis," (2) alters the birefringence of mitotic spindle in living cells, (3) delays the oral membranellar band regeneration of *Stentor*,¹¹ and (4) binds to vinblastine precipitated crystals of tubulin in competition with colchicine.¹² Inspection of molecular

(11) S. Banerjee and L. Margulis, *Exp. Cell Res.*, 78, 314 (1973).

(12) J. Bryan, *Biochemistry*, 11, 2611 (1972).

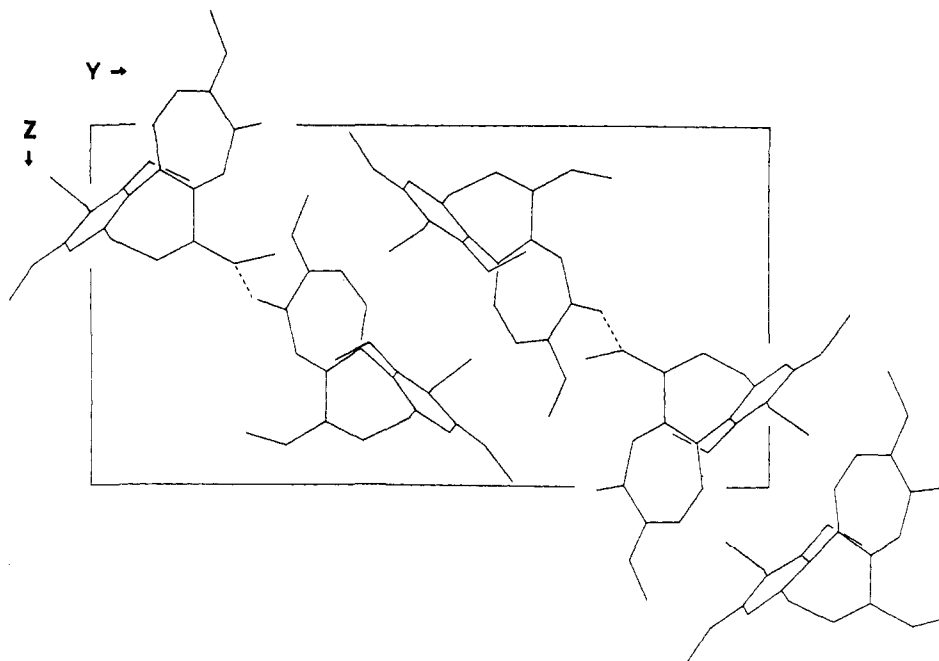


Figure 1. The crystal structure viewed along x . The dashed line indicates a hydrogen bond.

Table III. Observed Interatomic Distances^a

Atoms	Distance, Å	Atoms	Distance, Å
C(1)–O(1)	1.25	C(10)–C(11)	1.37
C(1)–C(2)	1.45	C(11)–C(12)	1.40
C(1)–C(7)	1.47	C(11)–O(5)	1.39
C(2)–O(2)	1.36	C(12)–O(4)	1.38
C(2)–C(3)	1.37	C(12)–C(13)	1.39
C(3)–C(4)	1.42	C(13)–O(3)	1.38
C(4)–C(5)	1.38	C(14)–C(15)	1.55
C(5)–C(6)	1.42	C(15)–C(16)	1.56
C(5)–C(8)	1.50	C(16)–N	1.45
C(6)–C(7)	1.38	C(17)–N	1.50
C(6)–C(16)	1.55	C(18)–O(3)	1.41
C(8)–C(9)	1.39	C(19)–O(4)	1.46
C(8)–C(13)	1.39	C(20)–O(5)	1.45
C(9)–C(10)	1.40	C(21)–O(2)	1.43
C(9)–C(14)	1.56		

^a Standard deviations are approximately 0.01 Å for each bond.

models reveals these molecules to have similar shapes. Three points of similarity are labeled a, b, and c, respectively, in the structural diagrams. Point a refers to a group capable of providing the hydrogen atom in a hydrogen bond, –NH in II and –OH in III. Point b refers to an ether oxygen atom, methoxy in II and cyclic in III. The a–b distance (O···N in II, O···O in III) is 6.55 Å in II and 6.27 Å in III. Point c refers to a methoxy group on a benzene ring with a–c = 6.32 Å in II and varying (in crystals) from 6.6 to 7.3 Å in III. In comparing these structures remember that while colcemid is quite rigid, the phenyl group of podophyllo-toxin may rotate about the bond attaching it to the rest of the molecule. The crystal structure of a 2'-bromopodophyllotoxin¹³ from which the interatomic distances above are obtained, and the related 5'-deme-thoxy- β -peltatin A methyl ether,¹⁴ shows the phenyl group approximately perpendicular to the mean plane of the rest of the molecule but the group might easily

(13) T. J. Petcher, H. P. Weber, M. Kuhon, and A. von Wartburg, *J. Chem. Soc., Perkin Trans. 2*, 288 (1972).

(14) R. B. Bates and J. B. Wood, *J. Org. Chem.*, 37, 562 (1972).

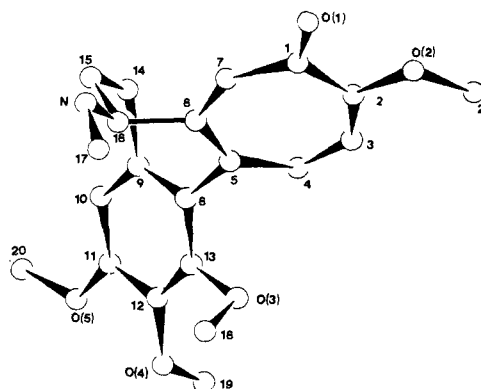


Figure 2. One molecule of colcemid viewed along the y axis. Atoms without element symbol are carbon.

Table IV. Bond Angles^a

Atoms	Angle, deg	Atoms	Angle, deg
C(2)–O(2)–C(21)	120	C(5)–C(8)–C(13)	122
C(13)–O(3)–C(18)	115	C(9)–C(8)–C(13)	118
C(12)–O(4)–C(19)	112	C(8)–C(9)–C(10)	122
C(11)–O(5)–C(20)	119	C(8)–C(9)–C(14)	119
C(16)–N–C(17)	113	C(10)–C(9)–C(14)	120
O(1)–C(1)–C(2)	119	C(9)–C(10)–C(11)	119
O(1)–C(1)–C(7)	117	O(5)–C(11)–C(10)	123
C(2)–C(1)–C(7)	124	O(5)–C(11)–C(12)	115
O(2)–C(2)–C(1)	110	C(10)–C(11)–C(12)	121
O(2)–C(2)–C(3)	122	O(4)–C(12)–C(11)	120
C(1)–C(2)–C(3)	128	O(4)–C(12)–C(13)	121
C(2)–C(3)–C(4)	131	C(11)–C(12)–C(13)	119
C(3)–C(4)–C(5)	130	O(3)–C(13)–C(8)	120
C(4)–C(5)–C(6)	127	O(3)–C(13)–C(12)	118
C(4)–C(5)–C(8)	115	C(8)–C(13)–C(12)	122
C(6)–C(5)–C(8)	118	C(9)–C(14)–C(15)	111
C(5)–C(6)–C(7)	128	C(14)–C(15)–C(16)	112
C(5)–C(6)–C(16)	116	N–C(16)–C(6)	118
C(7)–C(6)–C(16)	116	N–C(16)–C(15)	107
C(1)–C(7)–C(6)	132	C(6)–C(16)–C(15)	109
C(5)–C(8)–C(9)	121		

^a Standard deviations are approximately 1.0°.

change orientation to accommodate a receptor. Interaction with tubulin might take place *via* hydrogen bonding with specificity occurring because the ether oxygen¹⁵ can only hydrogen bond to an atom with hydrogen -NH or -OH.¹⁶ Hydrogen bonding allows wide latitude in configuration. For example, O-H...O distances¹⁷ may vary from about 2.5 to about 3.0 Å. Thus exact matching of distances in receptor and drug may not be necessary, and drugs where these distances vary may still be effective.

Other drugs which partially mimic colchicine are vinblastine and melatonin¹¹ which both fit criteria 1, 2, and 3 above. Vinblastine¹⁸ contains a methoxy group 6.0 Å away from -OH which could correspond to either the 6.6- or 5.6-Å distance in colcemid. Melatonin¹⁹ contains two -NH groups, one of them 5.5 Å

(15) K. B. Birnbaum, *Acta Crystallogr., Sect. B*, **28**, 1551 (1972).

(16) It is remarkable that so many natural products, *e.g.*, mescaline, reserpine, and brucine, contain methoxy groups. The ether group is fairly inert but may gain its usefulness from its ability to hydrogen bond to -NH or -OH.

(17) W. C. Hamilton and J. A. Ibers, "Hydrogen Bonding in Solids," W. A. Benjamin, New York, N. Y., 1968.

(18) J. W. Moncrief and W. N. Lipscomb, *Acta Crystallogr.*, **21**, 322 (1966).

(19) Interatomic distances in melatonin were calculated from the crystal structures of the related compounds, 5-methoxy-(*N,N*)-dimethyl-

from methoxy and the other found in crystals to be 7.1–7.9 Å from methoxy depending upon the conformation of the side chain. Inspection of models shows that the latter could change conformation and nicely match the 6.6-Å colcemid distance with good matching of the orientation of the -NH groups.

Acknowledgment. This work was supported in part by a grant from the National Science Foundation. I am indebted to Professor R. Rosenstein and Dr. H. Berman for their help with the Syd Hall method, to Professor L. Margulis for suggestions, and to the staff of the University of Massachusetts Research Computing Center.

Supplementary Material Available. A listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche, referring to code number JACS-74-899.

tryptamine hydrochloride [G. Falkenberg and D. Carlström, *Acta Crystallogr., Sect. B*, **27**, 411 (1971)] and 5-hydroxy-(*N,N*)-dimethyltryptamine [G. Falkenberg, *ibid.*, **28**, 3219 (1972)].

The Dielectric Increments of Amino Acids

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Abstract: The dielectric increments for the first ten members of the homologous series of α,ω -amino acids, together with those for a number of rigid or "pseudorigid" molecules, have been calculated from dielectric constants in aqueous solution. The results suggest that the flexible molecules exist in their most extended conformations and that solvent effects contribute significantly to the observed dielectric increments.

The importance of the conformational behavior of hydrophobic chains in aqueous media has become apparent in a variety of fields.^{1–6} In particular, the understanding of processes in biological and biomimetic systems requires clear descriptions of the preferred conformational states of these chains. Breslow has demonstrated very strikingly the criteria required in natural systems to transform, for example, stearic acid into oleic acid, introducing a double bond at a specific point in a homogeneous methylene chain.⁷ Such specificity is clearly dependent on the predominant conformation(s) of the aliphatic chain. The ubiquity of amino acids in biological systems has resulted in a large

body of work on their conformational behavior.^{8,9} The physiological activity of these compounds is dependent upon the conformations they are able to assume.^{10–13} In this paper we report the results (and interpretations thereof in terms of chain conformations) of a study of the dielectric constants of aqueous solutions of a larger series of α,ω -amino acids than has previously been examined.

Measurements of the dielectric constants of aqueous solutions of amino acids provided, in large measure, the evidence that such molecules exist in water as dipolar ions or zwitterions.¹⁴ Although at the time at which these studies were carried out there was no exact theory to interpret the dielectric constants of polar liquids,

(1) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969.

(2) J. L. Kavanau, "Structure and Function in Biological Membranes," Vol. 1, Holden-Day, San Francisco, Calif., 1965.

(3) W. D. Stein, "The Movement of Molecules Across Cell Membranes," Academic Press, London, 1967.

(4) H. Tompa, "Polymer Solutions," Butterworths, London, 1956.

(5) L. S. Sandell and D. A. I. Goring, *Macromolecules*, **3**, 50 (1970).

(6) S. Patai, "Chemistry of Carboxylic Acids and Esters," Interscience, New York, N. Y., 1969.

(7) R. Breslow, *Chem. Soc. Rev.*, **1**, 553 (1972).

(8) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. I, Wiley, New York, N. Y., 1961, pp 435–522.

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(10) D. R. Curtis and J. C. Watkins, *J. Neurochem.*, **6**, 117 (1960).

(11) D. R. Curtis and J. C. Watkins, *Pharmacol. Rev.*, **17**, 347 (1965).

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(13) N. M. van Gelder, *Can. J. Physiol. Pharmacol.*, **49**, 513 (1971).

(14) J. Wyman, *Chem. Rev.*, **19**, 213 (1936).